

Title: Amygdala lesions selectively impair familiarity in recognition memory

Anja Farovik, Ryan Place, Danielle Miller and Howard Eichenbaum

Supplementary information

Generating the ROC function

The model assumes that both recollection and familiarity contributes independently to recognition performance. The degree to which recollection and familiarity is used during recognition is dependent on the relationship between all 5 data points (i.e., confidence levels) that make up the ROC curve. According to the dual-process model of recognition memory, the asymmetry of the ROC in probability space (reflected prominently in an above-zero Y-intercept) indexes the contribution of recollection (R), whereas the degree of curvilinearity (reflected in the degree of “bowing” of the ROC curve) measures the contribution of familiarity (d') (Yonelinas and Parks, 2007). The higher the value is above zero on the y-intercept, the greater the contribution of recollection to recognition. The larger the “bowing” of the ROC curve, the greater the contribution of familiarity to recognition. A curvilinear ROC in probability space generally becomes linear in z -space and a linear ROC in probability space generally exhibits a U-shape in z -space.

Model equations:

$$1. P ('old'|old) = R + (1-R) F_{old} \quad (F_{old} = \Phi (d'/2 - c_i))$$

$$2. P ('old'|new) = F_{new} \quad (F_{new} = \Phi (-d'/2 - c_i))$$

3.

The variable d' reflects the distance between the equal-variance Gaussian strength distributions for old and new items, Φ is the cumulative response function, and c_i is response criterion at point i .

Statistical analysis

Using a least-square algorithm, ROC curves were created from the raw scores of hits and false alarms to estimate the contribution of recollection and familiarity to recognition performance. In order to confirm the curvilinearity and symmetry of the ROC functions, polynomial quadratic and linear regression analyses were performed on the hit and false alarm rates transformed into z -scores. A small-sample t -test was used to determine whether the obtained z -ROC quadratic coefficient differed significantly from 0 (if quadratic coefficient = 0, the equation becomes linear) in lesioned animals. Independent samples t -tests were used to determine any between group differences in the model's parameter estimates, calculated as the mean of each rat's individual R and d' estimate, whereas repeated measures ANOVA was used to identify an interaction between group and the model's R and d' parameter estimates. The hit and false alarm rates were analyzed separately using repeated measures ANOVA across bias levels (1-5) as within-subjects factor, and group as the single between-subjects factor (control vs. amygdala lesion).

Familiarity estimates (conversion of d' to F). The parameter estimates in the bar graphs (insets) are a result of performing the ROC analysis on the hits and false alarms for each bias in each individual rat instead of a group analysis. Performing the ROC analysis on each rat's data means that we get an R and d' value from each individual animal (which is used for data analysis), and the parameter estimates in the bar graph is an average of rats' R and F values. Whereas the average seen for recollection (R) in the insets is a direct reflection of the data obtained for this variable, F (familiarity) is a conversion of the d' values obtained to facilitate comparison with recollection (R), which is measured as probability. The F estimates are

obtained by calculating the probability of a hit given a false alarm rate of 0.03669, such that the familiarity estimates (F) would be close to the value of preoperative recollection estimates for the control group (~ 0.23 ; see Fig. 2a). The equation used to determine the probability of a hit for a given probability of false alarm is NORMINV (probability, mean, standard deviation) with the following values in the equation: $\text{NORMINV}(0.03669, 0, 1) = -1.79046$ $\leftarrow c$ is the value at which the area to the left of the Hit curve is equal to a given FA rate. To obtain the F value for each animal, the resulted value of the NORMINV is tested using the NORMDIST function, which gives the probability that a number falls at or below a given value of the normal distribution = $\text{NORMDIST}(x, \text{mean}, \text{standard deviation}, \text{cumulative})$ where the mean is the rat's d' value. This gives us $\text{NORMDIST}(-1.79046, d', 1, \text{TRUE}) = \text{familiarity estimate (F)}$ for the animal.

Unequal-variance signal-detection model (UVSD). To determine whether the unequal-variance signal detection model could also detect a recognition deficit following amygdala damage, we compared the d' estimates obtained using this model in controls versus animals with amygdala damage. This analysis did not reveal a group difference ($t_{11} = 1.03, P = .32$), indicating that the single-process model is insensitive to the effects of amygdala damage on recognition performance. Analysis of the z-ROCs in amygdala lesioned animals (see main results), where recognition relied on recollection due to impaired familiarity, revealed a U-shaped function in z-space, which is inconsistent with the UVSD model's predictions of a linear z-space ROC function and supports a dual-process model of recognition.

Tests for response bias. A lesion-produced response bias towards or away from responding to test stimuli would be reflected in a group difference in the overall rate of “old” and “new” responses to the target stimuli. We measured the overall rate of responding to the test stimuli as a combination of correct rejections (correct “new” responses) and misses (incorrect “new” responses) to test stimuli, both of which involve digging in the test cup. This analysis revealed no significant difference ($t_{11} = 1.22$, $p = 0.25$) between controls (46%) and amygdala-lesion subjects (40%) in the overall tendency to dig in test cups (see Supplementary figure).

Supplementary methods

Animals. Subjects were thirteen male Long-Evans rats (Charles River, MA) weighing between 225-250 grams at the start of the experiment. All animals were single housed and maintained on a 12-hr light/dark cycle (lights on 8.00 am - 8.00 pm). Behavioral training and testing were conducted during the light phase. Animals were kept at approximately 85 % of their free feeding body weight and had free access to water in the home cage. Procedures were conducted according to the requirements set by the National Institutes of Health (NIH) and Boston University Institutional Animal Care and Use Committee (IACUC).

Materials and apparatus. Behavioral training and testing were carried out in the home cage (44x21x20 cm). The materials consisted of transparent Nalgene cups (VWR) in three different sizes (4.0, 6.5 and 8.5 cm high) with an internal diameter of approx. 6.5 cm. A selection of forty different odors was used in the study. The cups were attached to a black Plexiglas platform using Velcro (VWR) before they were lowered into the cage. Cereal Froot Loops (Kellogg's) were used as reinforcement.

Behavioral protocol. Animals were trained in successive stages. Initially, rats were trained to dig for reward (one Froot Loop) buried in a cup filled with unscented sand. Once the animals had learned to dig reliably to retrieve the reward, they were introduced to an odor recognition task where each trial consisted of a sample and a test phase. In the sample phase, animals were presented with a cup filled with sand and one of the forty odors in the front of the cage. Following a 1 min delay, two cups, each with a different odor (one old and one new), were presented consecutively and in a pseudo-randomized order. For new responses, $\frac{1}{4}$ Froot Loop was buried in the cup and two whole Froot Loops were provided in the back of the cage for correct ‘old’ responses. Trials that required the same response (‘new’ or ‘old’) did not occur more than three times in a row during the test phase. For new trials, responses were defined as the animal moving the sand with the forepaw. Once the animals reached a criterion of 80 percent correct across two consecutive sessions (each session consisted of 10 trials; 10.8 ± 2.7 s.d. sessions to reach criterion), each session consisted of a series of five sample odors, then a 1 min delay, then five old and five new odors in pseudo-random order during the test phase. After the same performance criterion was reached (13.3 ± 4.4 s.d. sessions to criterion), each session consisted of a set of ten sample odors and ten old and ten new test odors, and the delay was increased to ten minutes (11.2 ± 2.9 s.d. sessions to criterion). Odors were selected such that all 40 were used equally often, and different cups were used for odors when they appeared in both the sample and test phases. Finally, five distinct response biases were introduced by manipulating height of the cup, and therefore the effort required to dig for reward, and the amount of reward obtained for correct “old” and “new” responses. Animals were habituated to the different response biases, and ROC analysis was performed on the following 30 pre-

operative and 30 post-operative sessions (six sessions per bias level), using a 30 min delay period between study and test.

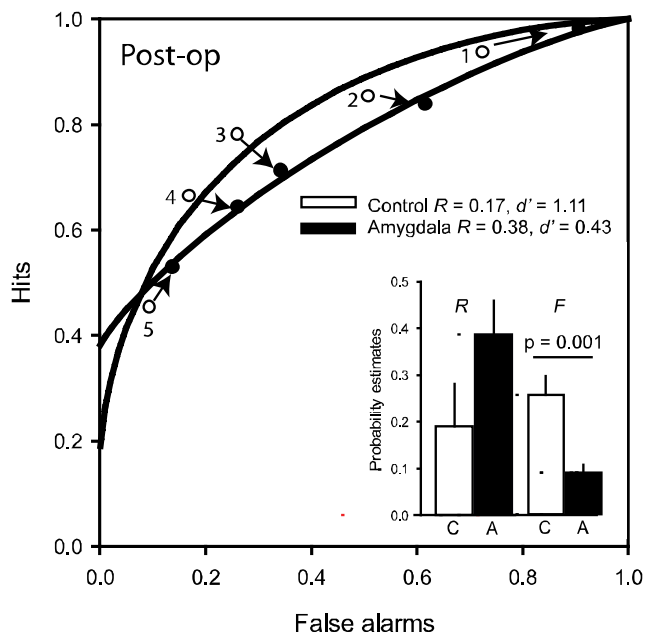
Surgery. Anesthesia was induced by inhalation of 5% Isoflurane (Webster Veterinary Supply, Inc, MA) in oxygen and was maintained at 2 – 2.5% throughout surgery. Rats were placed in a stereotaxic frame (Kopf, Tujunga, CA) and an incision was made along the midline to expose the skull. Using a 1 μ l Hamilton syringe, seven rats were given 4 injections each of 0.15 μ l/site of 0.06 M ibotenic acid (Tocris Cookson Inc., MO) into the basolateral amygdala (BLA). Each injection of 0.15 μ l /site was infused at a rate of 0.1 μ l/min and was made using a microsyringe pump (World Precision Instruments, Inc, FL). The needle was left *in situ* for 3 minutes after injection to allow for diffusion. Lesions were made using co-ordinates from the atlas of Paxinos & Watson (1998): from bregma, AP - 2.28 mm, ML \pm 4.6 mm, DV – 7.9 mm (from dura); and AP - 2.76 mm, ML \pm 4.6 mm, DV – 7.8 mm. Diazepam (5mg/ml; Webster Veterinary Supply, Inc, MA) was given intramuscularly (i.m.) immediately after surgery to prevent convulsions. Another six rats (controls) underwent the same procedure as the lesion group but saline was infused instead of ibotenic acid. After surgery, general health was monitored until they recovered and returned to testing, approximately 1 week after surgery.

Histology. After completion of behavioral testing, rats were overdosed with 0.8 ml Sodium Pentobarbital (Fort Dodge Animal Health, IA). Animals were then perfused transcardially with 0.9 % saline, followed by 10% formalin (VWR, PA), and the brains were removed and placed in a 20% sucrose solution until processed. Using a cryostat (Reichert-Jung, Kramer Scientific Corp.) brains were cut into 50 μ m coronal sections and mounted onto pre-subbed glass slides, and stained with cresyl violet to determine the location and extent of the lesion.

Anatomical observations. Three animals were excluded from analysis because the lesions did not comprise the basolateral amygdala, leaving seven animals in the lesioned group and six animals as controls. All animals received substantial damage to the central amygdala nuclei, and thus, this region was included in the histological quantification. Histological quantification showed that the lesioned animals lost, on average, 44 % of the total volume of the central and basolateral amygdala combined at - 2.2 mm anterior to bregma; 63% of the total volume was damaged at - 2.7 mm from bregma, whereas 68 % was damaged at the most posterior coordinates (- 3.2 mm from bregma). Six out of the lesioned animals sustained some damage to the ventral portion of the caudate putamen and in three animals damage to the ventral globus pallidus was evident. Damage was also evident in the medial sector of the amygdala and the endopiriform nucleus in all animals.

Paxinos G, Watson C. 1998. The rat brain in stereotaxic coordinates, 4th ed. Academic Press, San Diego, CA.

Yonelinas, AP, Parks, CM. 2007. Receiver operating characteristics (ROCs) on recognition memory: a review. Psychol Bull 133, 800-832.



Supplementary Fig 1. Post-operative recognition performance, showing the direction of hits and false alarms after lesions to the amygdala. Amygdala lesions did not cause a shift in the general tendency to elicit “old” versus “new” responses.